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VACCINE COMPOSITIONS COMPRISING LIVE BACTERIAL VECTORS FOR PROTECTION AGAINST YERSINIA PESTIS INJECTION

Abstract:

Novel DNA constructs are provided that are capable of transforming microorganisms such that they can be used as live or attenuated vaccines which induce such immune response at mucosal surfaces. Further provided are such transformed microorganisms per se and vaccine compositions containing them. Preferred constructs of the invention are capable of transforming microorganisms such that they express F1 based protein while retaining a capability to establish themselves in human or animal gut environment. Several constructs have been identified that are capable of transforming gut dwelling organisms such as <u>S. typhimurium</u> or <u>S. typhi</u> to enable F1 antigen production, but most of these affect the organism such that it can no longer function effectively in the gut, at least in so far as it cannot express the antigen e.g. being unstable and losing plasmid.

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(57) Abstract

Novel DNA constructs are provided that are capable of transforming microorganisms such that they can be used as live or attenuated vaccines which induce such immune response at mucosal surfaces. Further provided are such transformed microorganisms per se and vaccine compositions containing them. Preferred constructs of the invention are capable of transforming microorganisms such that they express F1 based protein while retaining a capability to establish themselves in human or animal gut environment. Several constructs have been identified that are capable of transforming gut dwelling organisms such as S. typhimurium or S. typhi to enable F1 antigen production, but most of these affect the organism such that it can no longer function effectively in the gut, at least in so far as it cannot express the antigen e.g. being unstable and losing plasmid.

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VACCINE COMPOSITIONS COMPRISING LIVE BACTERIAL VECTORS FOR PROTECTION AGAINST YERSINIA PESTIS INJECTION.

The present invention relates to novel vaccines for provision of protection against infection with the organism Yersinia pestis (Y. pestis) and to compositions containing them. Particularly provided are parentally and orally active vaccines capable of offering protection against bubonic and pneumonic plague, particularly by induction of mucosal immunity in both humans and other animals.

Y. pestis is the highly virulent causative organism of plague in a wide range of animals, including man. Infection with this organism results in a high rate of mortality. Studies have shown that the high virulence is due to a complex array of factors encoded by both the chromosome and three plasmids, including the Lcr genes (see Straley, (1991) Microb. Pathogen 10: pp87-91), a fibrinolysin (Sodeinde & Goguen, (1988) Infec. Immun 56: pp2743-2748), and a capsule.

The capsule surrounding Y. pestis cells is composed of a protein -polysaccharide complex, the protein component of which is known as Fraction 1 (F1) (see Baker et al (1952) J. Immunol 68: pp131-145) which is only fully expressed at 37°C. This complex confers resistance to phagocytosis, possibly by forming aqueous pores in the membranes of phagocytic cells (Rodrigues et al (1992) J. Med. Biol. Res. 25: pp75-79). Detection of antibodies to F1 is the basis of standard serological tests for the surveillance and diagnosis of plague as infected animals produce a strong humoral response to the antigen (Shepherd et al (1986) J. Clin. Microbiol. 24: pp1075-1078); Williams et al, (1982) Bull. World Health Organ. 64: pp745-752).

The current whole cell vaccines available for prevention of plague are highly heterogeneous, resulting in side effects which make them unsuitable for widespread use (Reisman (1970) J. Allergy 46: pp49-55); Meyer et al (1974) J. Infect. Dis. 129: S13-S18);

Marshall et al (1974) J. Infect. Dis. 129: S19-S25). F1 has been proposed as being the primary immunogen in whole cell vaccines (Williams et al (1980) Bull. World. Health Organ. 58: pp753-756; Chen et al, (1976) J. Infect. Dis. 133: pp302-309) and may therefore be a suitable candidate on which to base an improved vaccine against plague.

The caf1 gene encoding F1 antigen has been cloned and sequenced (Galyov et al (1990) FEBS Letters 277: pp230-232) and recombinant F1 expressed and purified from E.coli induced a protective response in BALB/c mice sufficient to protect against challenge with 10^5 virulent plague bacilli (Simpson et al (1990) Am. J. Trop. Med. Hyg. 43(4): pp389-396). Such resistance to infection by Y. pestis is correlated with high titres of F1 antibody.

It is known to orally administer genetically engineered organisms which express antigenic proteins for the purpose of inducing antigen production (EP 0474891) and it is further known that use of such route may result in mucosal immunity (Cardenas & Clements (1992) Clin. Microbiol Rev 5 (3) pp328-342 and (1992) Vaccine 10 (4) pp263). Furthermore, it has been reported that attenuated bacteria have been prepared that are protective against enteric diseases, including genus Yersinia (US 6865709).

The current vaccine for plague is the Cutter vaccine which comprises formaldehyde killed plague bacilli and is administered to the body by intramuscular injection. However, parenteral immunisation, although effective in inducing systemic immunity, does not effectively induce mucosal immunity (McGhee et al, (1992) Vaccine 10, 75-88). So far no Y. pestis vaccine capable of producing a protective immune response at mucosal surfaces has been developed.

The present inventors have now provided recombinant DNA constructs that when incorporated into DNA of a microorganism, particularly

of a human or animal gut colonising microorganism, are capable of transforming it such that it is able to express a protein which produces a protective immune response against Yersinia pestis in the human or animal body when the gut colonising microorganism is administered by oral routes.

Preferred forms of the present invention provide such DNA constructs that transform such a microorganism while allowing it to maintain its ability to colonise the human or animal gut and thus provide sustained administration, ie. by exposure of antigen to the human or animal body immune system, preferably with systemic invasion of the human or animal body.

Further provided are vectors eg.plasmids, containing the DNA constructs of the invention, that are capable of transforming a human or animal gut colonising microorganism such that it is capable of expressing a protein which produces a protective immune response against Yersinia pestis in a human or animal body when the microorganism is administered by oral or parenteral routes, preferably allowing the microorganism to maintain ability to colonise the human or animal gut, and preferably subsequently systemically to invade the body.

Still further provided are microorganisms, preferably human or animal gut colonising microorganisms, transformed with a vector containing recombinant DNA, eg. a plasmid containing recombinant DNA, according to the invention such that it is enabled to express a protein which produces a protective immune response against Yersinia pestis in a human or animal body when the microorganism is administered by oral or parenteral routes, and preferably allows the microorganism to maintain its ability to colonise the human or animal gut, and preferably invade systemically. The protective response provided preferably includes protection at mucosal surfaces.

One preferred recombinant DNA, and plasmids comprising it, are

characterised in that they comprise a lacZ promoter in frame with a sequence encoding for all or part of the caf1 antigen. Still further preferred recombinant DNA utilises a caf1R positive regulator derived from the F1 operon itself. It is further preferred that the caf1M fragment is included for the purposes of assisting export of mature protein through the cell wall of the host organism, ie. the transformed cell and/or the caf1A fragment that encodes proteins thought to be important in anchoring the F1 subunit to the cell wall.

Where the recombinant DNA includes the lacZ promoter it is preferably incorporated into a vector such as pUC18 that has the all or part of the caf1 encoding sequence inserted in frame with the lacZ promoter

Obviously the microorganisms of the invention are preferably attenuated microorganisms not capable of causing disease in humans or animals, eg. such as the Salmonella aro A or aro C mutants, preferably of species Salmonella typhimurium or a Salmonella typhi. Preferred vaccine compositions are provided as such microorganisms together with a pharmaceutically acceptable carrier, eg. saline or buffer.

Attenuated microorganisms such as S. typhimurium have been well characterised as carriers for various heterologous antigens (Curtiss, (1990); New Generation Vaccines, Woodrow & Levine (eds) Marcel Dekker Inc. New York; Cardenas and Clements, (1992) ibid). Attenuation may be effected in a number of ways, such as by use of the aro A and/or aro C mutation approach (see Hosieth et al (1981) Nature 291, 238-239; Dougan et al (1986) Parasite Immunol 9, 151-160; Chatfield et al (1989) Vaccine 7, 495-498). Many other such attenuating deletions and mutations will be known for these and other microorganisms which will render them suitable for transformation with constructs of the present invention for the purposes of expressing vaccine proteins in the gut and/or gut colonisation in animals to be treated for Y.pestis, with systemic invasion and colonisation

following. For human vaccination attenuated S. typhi is the preferred microorganism.

A particularly preferred recombinant DNA, and plasmid or human or animal gut colonising microorganism incorporating it, encodes for or expresses all or part of the mature caf1 (F1) protein of Yersinia pestis. A particularly preferred recombinant DNA comprises a DNA sequence as described in SEQ ID No 4.

The inventors have determined the sequence of a still further preferred recombinant DNA which when included within suitable vectors within, or integrated directly into the chromosomal DNA of, gut dwelling microorganisms results in still stronger expression of protective F1, F1 fusion or F1 truncate proteins. This recombinant DNA is particularly characterised in that it it comprises the complete F1 operon including caf1R, a positive regulator of F1 expression; caf1M, encoding for the proposed chaperone sequence which assists in export of the F1 sub-units across the cell wall; caf1A, thought to encode a protein which anchors the F1 into the cell wall; all in addition to the caf1 gene encoding the F1 subunit or a truncate or fusion product thereof.

The method, constructs, microorganisms and vaccines of the invention will now be exemplified by way of illustration only by reference to the following Sequence listing, Figure and Examples. Still further embodiments will be evident to those skilled in the art in the light of these.

SEQUENCE LISTING:

SEQ ID No 1: is the sequence of a PCR primer oligonucleotide corresponding to the first 21 bases encoding for mature caf1 with an additional 5' region encoding for a SacI site.

SEQ ID No 2: is the sequence of a PCR primer oligonucleotide corresponding to the sequence of caf1 which encodes a 'stem loop' downstream of the termination codon with an added 5' region encoding SacI and AccI sites.

SEQ ID No 3: is that of a PCR primer oligonucleotide corresponding to an internal end region of the caf1 gene starting 107 bases downstream from the end of the first oligonucleotide.

SEQ ID No 4: is that of the pFGAL2a construct showing the fusion of the first few bases of the β-galactosidase sequence in the vector with caf1 minus its signal sequence and having a 5' tail including a Sac I restriction site; the sequence is shown up to the caf1 AACC 3' end with some vector bases.

SEQ ID No 5: is that of the protein encoded by pFGAL2a.

SEQ ID No 6: is that of pFSIG3a: including caf1 sequence encoding mature F1 expressed as a fusion with the E. coli LTB signal sequence encoded by the vector and having 5' tail including a SacI restriction site; the sequence shown to AACC at 3' end of caf1 and its adjoining vector bases.

SEQ ID No 7: is that of the protein encoded by pFSIG3a.

SEQ ID No 8: is that of pFORF1b: including the entire caf1 gene and having a 5' tail including a SacI restriction site; the sequence shown to TATAG downstream of the caf1 open reading frame. The two series encoded at the 5' end of the sequence are produced separately to the F1 fusion.

SEQ ID No 9: is that of the end of the first protein encoded by pFORF1b.

SEQ ID No 10: is that of the F1 fusion encoded by pFORF1b.

SEQ ID No 11: is that of primer FIOU2 used to amplify the F1 operon.

SEQ ID No 12: is that of primer M4D used to amplify the F1 operon.

SEQ ID No 13: is that of primer M3U used to amplify the F1 operon.

SEQ ID No 14: is that of primer FIOD2 used to amplify the F1 operon.

SEQ ID No 15: is that of a primer used with the primer of SEQ ID No 1 for preparation of pFSIG3a.

SEQ ID No 16: is that of a primer used with the primer of SEQ ID No 17 to produce pFORF1b.

SEQ ID No 17: is that of a primer used with the primer of SEQ ID No 16 to produce pFORF1b.

FIGURE:

Figure 1 shows schematic representations of the positions of F1 sequences in the constructs pFGAL2a, pFSIG3a and pFORF1b.

Figure 2 shows to relative positions of the primers SEQ ID No 11 to 14, the retriction enzyme sites and the caf1R, caf1M, caf1A and caf1 subunits.

EXAMPLES.

General methods: Y. pestis was grown aerobically at 28°C in Blood Agar Base broth, pH6.8, containing 15g/l proteose peptone, 2.5g/l liver digest, 5g/l yeast extract, 5g/l NaCl supplemented with 80ml 0.25% haemin in 1/100N NaOH. Strains of S. typhimurium used were

SL3261 and LB5010 as described by Hosieth & Stocker (1981) Nature (London) 291: p238-239 and Maskell et al (1987) Microb. Pathog. 2 pp211-221 respectively and these and E.coli JM109 were cultured and stored as described by Sambrook et al (1989) Molecular Cloning Manual.

Cloning of caf1: DNA was isolated from Y. pestis by the method of Marmur et al (1961) J. Mol. Biol. 3: pp 208-218. A DNA fragment encoding the open reading frame of caf1 minus its signal sequence was amplified from this using the polymerase chain reaction (PCR).

Oligonucleotides were prepared with a Beckman 200A DNA synthesiser for use in the PCR.

EXAMPLE 1: pFGAL2a construct:

Oligonucleotide GATCGAGCTCGGCAGATTTAACTGCAAGCACC (SEQ ID No 1) was synthesised corresponding to the first 21 bases of caf1 immediately following the nucleotides encoding the signal sequence with an additional 5' region encoding a SacI site and the complimentary oligonucleotide CAGGTCGAGCTCGTCGACGGTTAGGCTCAAAGTAG (SEQ ID No 2) corresponding to the sequence which encodes a putative 'stem loop' structure downstream of the caf1 termination codon with an added 5' region encoding SacI and AccI sites. A DNA fragment was obtained after 35 cycles of amplification (95°C, 15 secs; 50°C, 15 secs; 72°C, 30 secs using a Perkin Elmer 9600 GeneAmp PCR system). The fragment was purified, digested with SacI and AccI, ligated into a similarly digested pUC18 plasmid and transformed into E. coli JM109 by electroporation. Electroporation was carried out using a Biorad Gene Pulser with 0.2 cm cuvettes at 1.25kV, 25μF, 800Ohms with a time constant of 20.

A pFGAL2a colony containing the cloned caf1 gene was identified by PCR using an oligonucleotide TGGTACGCTTACTCTTGGCGGCTAT (SEQ ID No 3) corresponding to an internal region of the gene 128 to 153 nucleotides from the site identified as the signal sequence cleavage site (see

Galyov et al (1990)) and the SEQ ID No 2. A culture of the E.coli containing the pFGAL2a was grown at 37°C with shaking in Luria Broth containing 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 18 hours. Whole cell lysates and periplasmic and cytoplasmic fractions of the bacteria were prepared as described by Sambrook et al (1989).

SDS-PAGE and Western blotting: SDS-polyacrylamide gel electrophoresis (PAGE) and Western blotting were performed as described by Hunter et al (1993) Infec. Immun. 61. 3958-3965. Blots were probed with polyclonal antisera raised in sheep (B283) against killed Y. pestis (EV76 strain grown at 37°C) and bound antibody was detected with a horseradish peroxidase-labelled donkey anti-sheep IgG (Sigma).

Expression of F1 in S. typhimurium: The pFGAL2a plasmid was isolated using general techniques described in Sambrook et al (1989) Molecular Cloning; a Laboratory Manual. 2nd Edition. Cold Spring Harbour Laboratory, New York. Purified plasmid was electroporated into S. typhimurium LB5010 (restriction$^-$, modification$^+$) and methylated pFGAL2a was subsequently isolated from the LB5010 for electroporation into S. typhimurium SL3261 (aro A$^-$). Periplasmic and cytoplasmic fractions were prepared for SDS-PAGE and Western blotting as described above.

Stability of constructs: Five female Balb/c mice were inoculated intravenously with either $5x10^5$ or $5x10^7$ cfu S. typhimurium containing pFGAL2a in 200µl phosphate buffered saline. Control mice were inoculated similarly with S. typhimurium containing pUC18 with no insert. After 7 days the mice were killed by cervical dislocation and their livers and spleens removed. The organs were homogenised in 10ml phosphate buffered saline using a stomacher on maximum setting for 2 minutes and the homogenate was serially diluted in phosphate buffered saline and placed onto L agar or L agar containing 55µg ml^{-1} ampicillin.

Challenge of immunized mice: Male Balb/c mice were inoculated with three doses of S.typhimurium containing pFGAL2a, intravenously (i.v.) with $5x10^7$ cfu bacteria at 7 day intervals, or intragastrically (i.g.) by intubation with 1×10^{10} cfu on days 1, 7 and 21. Ampicillin was given subcutaneously for 5 days after each inoculation to stabilise the plasmid. Six weeks after the last dose five mice from both the i.g. and intravenously inoculated groups were bled then killed by cervical dislocation and their livers and spleens taken. Livers were homogenised and plated onto L-agar to confirm that all Salmonella had been cleared.

The remaining mice were challenged subcutaneously with $50LD_{50}$ of Y. pestis strain GB. Control mice were immunised i.v. and i.g. with S. typhimurium containing pUC18, intramuscularly with formaldehyde-killed plague vaccine (Cutter USP) or intraperitoneally with 10µg of purified F1 and survival rates assessed (see Table 1).

EXAMPLE 2: pFSIG3a construct:

Construct plasmids and S. typhimurium transformed by them were obtained using the method of Example 1 altered in so far as the PCR product was designed to be of SEQ ID No 2 after digestion with SacI. The digested product was ligated into SacI digested pFS2.2 giving the sequence SEQ ID No 5 located as shown in Figure I. This construct encodes for F1 which is expressed as a fusion with the E.coli LTB signal sequence. The primer used instead of SEQ ID No 1 was that of SEQ ID No 15.

Example 3: pFORF1b construct:

Construct plasmids and S. typhimurium transformed by them were obtained using the method of Example 1 altered in so far as the PCR primers were selected to produce an amplification product which after digestion with SacI/AccI consisted of SEQ ID No 6.

Primers used were of sequences SEQ ID NO 16 and SEQ ID No 17.

This construct incorporates the caf1 signal sequence in addition the mature protein sequence as shown in SEQ ID No 6 and Fig 1 which shows all bases downstream of the Sac I site used for digest.

Protection provided by intravenous injection and oral delivery of S.typhimurium transformed with plasmids containing various constructs of the invention and comparative protection afforded by direct administration of F1 and Cutter vaccines. S. typhimurium transformed as described above with either pFGAL2a, pFSIG3a, pFORF1b or unmodified pUC18 were administered to mice by intravenous or intragastric routes and compared in effect to intraperitoneal F1 and intramuscular Cutter vaccine as described above in Example 1.

TABLE 1. CHALLENGE OF MICE WITH 50 x MICE LD_{50} Y. PESTIS

IV or IM*TREATMENT	SURVIVORS	ORAL TREATMENT	SURVIVORS
S.typhimurium /pFSIG3a IV	10/10	S.typhimurium /pFSIG3a	1/10
S.typhimurium /pFGAL2a IV	9/10	S.typhimurium /pFGAL2a	9/10
S.typhimurium /pFORF1b IV	10/10	S.typhimurium /pFORFib	3/10
S.typhimurium /pUC18 IV	0/10	S.typhimurium /pUC18	0/5
F1 protein IP	6/8		
Cutter vaccine IM	9/10		

IV = intravenous IM = intramuscular IP = intraperitoneally

EXAMPLE 4: F1 operon construct:

Attempts to PCR replicate the entire F1 operon as one piece were unsuccessful, so a strategy was developed whereby it was amplified using PCR to produce two discrete fragments using primer pairs (A) of SEQ ID No 11 and 12 and (b) of SEQ No 13 and 14 respectively to produce fragments of 3.36kb and 1.89kb from Y. pestis MP6 template DNA. Marmur extract of DNA was used without $CsCl_2$ purification. The PCR cycle conditions used were 96°C for 30 seconds, 57°C for 30 seconds and 72°C for 1 minute; total of 30 cycles.

These two fragments were digested using Nhe1 and joined together. This fused fragment, encoding the full length operon (5.25kb), was digested with EcoR1 and Sal1 and then cloned into a number of vectors. When this fragment was cloned into pBR322 and expressed in E. coli, S. typhimurium LB5010 or SL3261 instability of the recombinant plasmid was noted. To circumvent this problem the operon was cloned into plasmid pLG339, a low copy number plasmid km^R. The entire F1 operon was also been inserted into AroC gene on the chromosome of S. typhimurium using vector pBRD1084.

The positions of the primers, restriction sites and caf1R to caf1 are shown in Figure 2.

Full protection (4/4) of mice challenged with with 10^4 lethal doses of Y. pestis was provided on administration of these transformed organisms.

SEQUENCE LISTING

1) GENERAL INFORMATION:

(i) APPLICANT:
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(E) COUNTRY: UNITED KINGDOM (GB)
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(C) CITY: SALISBURY
(D) STATE: WILTSHIRE
(E) COUNTRY: UNITED KINGDOM (GB)
(F) POSTAL CODE (ZIP): SP4 OJQ

(ii) TITLE OF INVENTION: VACCINE COMPOSITIONS
(iii)NUMBER OF SEQUENCES: 14
(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release @1.0, Version @1.25 (EPO)
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9326425.7
(B) FILING DATE: 24-DEC-1993

(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GATCGAGCTC GGCAGATTTA ACTGCAAGCA CC 32

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

CAGGTCGAGC TCGTCGACGG TTAGGCTCAA AGTAG 35

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A)ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

TGGTACGCTT ACTCTTGGCG GCTAT 25

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A)LENGTH: 541 base pairs
(B)TYPE: nucleic acid
(C)STRANDEDNESS: double
(D)TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A)ORGANISM: Yersinia pestis
(ix) FEATURE:
(A)NAME/KEY: CDS
(B)LOCATION: 2..454
(ix) FEATURE:
(A)NAME/KEY: misc recomb
(B)LOCATION: 1..6
(ix) FEATURE:
(A)NAME/KEY: misc_recomb
(B)LOCATION: 536..541
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
G AGC TCG GCA GAT TTA ACT GCA AGC ACC ACT GCA ACG GCA ACT CTT     46
  Ser Ser Ala Asp Leu Thr Ala Ser Thr Thr Ala Thr Ala Thr Leu
    1               5                  10                  15

GTT GAA CCA GCC CGC ATC ACT ATT ACA TAT AAG GAA GGC GCT CCA ATT   94
Val Glu Pro Ala Arg Ile Thr Ile Thr Tyr Lys Glu Gly Ala Pro Ile
                20                  25                  30

ACA ATT ATG GAC AAT GGA AAC ATC GAT ACA GAA TTA CTT GTT GGT ACG  142
Thr Ile Met Asp Asn Gly Asn Ile Asp Thr Glu Leu Leu Val Gly Thr
            35                  40                  45

CTT ACT CTT GGC GGC TAT AAA ACA GGA ACC ACT AGC ACA TCT GTT AAC  190
Leu Thr Leu Gly Gly Tyr Lys Thr Gly Thr Thr Ser Thr Ser Val Asn
        50                  55                  60

TTT ACA GAT GCC GCG GGT GAT CCC ATG TAC TTA ACA TTT ACT TCT CAG  238
Phe Thr Asp Ala Ala Gly Asp Pro Met Tyr Leu Thr Phe Thr Ser Gln
    65                  70                  75

GAT GGA AAT AAC CAC CAA TTC ACT ACA AAA GTG ATT GGC AAG GAT TCT  286
Asp Gly Asn Asn His Gln Phe Thr Thr Lys Val Ile Gly Lys Asp Ser
80                  85                  90                  95

AGA GAT TTT GAT ATC TCT CCT AAG GTA AAC GGT GAG AAC CTT GTG GGG  334
Arg Asp Phe Asp Ile Ser Pro Lys Val Asn Gly Glu Asn Leu Val Gly
                100                 105                 110

GAT GAC GTC GTC TTG GCT ACG GGC AGC CAG GAT TTC TTT GTT CGC TCA  382
Asp Asp Val Val Leu Ala Thr Gly Ser Gln Asp Phe Phe Val Arg Ser
            115                 120                 125
```

```
ATT GGT TCC AAA GGC GGT AAA CTT GCA GCA GGT AAA TAC ACT GAT GCT  430
Ile Gly Ser Lys Gly Gly Lys Leu Ala Ala Gly Lys Tyr Thr Asp Ala
        130                 135                 140

GTA ACC GTA ACC GTA TCT AAC CAA TAATCCATAT AGATAATAGA TAAAGGAGGG 484
Val Thr Val Thr Val Ser Asn Gln
    145                 150

CTATTATGCC CTCCTTTAAT ATTTATGAAT TATCCTACTT TGAGCCTAAC CGTCGAC   541
```

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A)LENGTH: 151 amino acids
(B)TYPE: amino acid
(D)TOPOLOGY: linear
(ii)MOLECULE TYPE: protein
(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Ser Ser Ala Asp Leu Thr Ala Ser Thr Thr Ala Thr Ala Thr Leu Val
 1               5                  10                  15

Glu Pro Ala Arg Ile Thr Ile Thr Tyr Lys Glu Gly Ala Pro Ile Thr
            20                  25                  30

Ile Met Asp Asn Gly Asn Ile Asp Thr Glu Leu Leu Val Gly Thr Leu
        35                  40                  45

Thr Leu Gly Gly Tyr Lys Thr Gly Thr Thr Ser Thr Ser Val Asn Phe
    50                  55                  60

Thr Asp Ala Ala Gly Asp Pro Met Tyr Leu Thr Phe Thr Ser Gln Asp
65                  70                  75                  80

Gly Asn Asn His Gln Phe Thr Thr Lys Val Ile Gly Lys Asp Ser Arg
                85                  90                  95

Asp Phe Asp Ile Ser Pro Lys Val Asn Gly Glu Asn Leu Val Gly Asp
            100                 105                 110

Asp Val Val Leu Ala Thr Gly Ser Gln Asp Phe Phe Val Arg Ser Ile
        115                 120                 125

Gly Ser Lys Gly Gly Lys Leu Ala Ala Gly Lys Tyr Thr Asp Ala Val
    130                 135                 140

Thr Val Thr Val Ser Asn Gln
145                 150
```

(2) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..455
(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..6
(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 536..541
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
GA GCT CCC GCA GAT TTA ACT GCA AGC ACC ACT GCA ACG GCA ACT CTT  47
   Ala Pro Ala Asp Leu Thr Ala Ser Thr Thr Ala Thr Ala Thr Leu
     1               5                  10                  15

GTT GAA CCA GCC CGC ATC ACT ATT ACA TAT AAG GAA GGC GCT CCA ATT  95
Val Glu Pro Ala Arg Ile Thr Ile Thr Tyr Lys Glu Gly Ala Pro Ile
                20                  25                  30

ACA ATT ATG GAC AAT GGA AAC ATC GAT ACA GAA TTA CTT GTT GGT ACG 143
Thr Ile Met Asp Asn Gly Asn Ile Asp Thr Glu Leu Leu Val Gly Thr
            35                  40                  45

CTT ACT CTT GGC GGC TAT AAA ACA GGA ACC ACT AGC ACA TCT GTT AAC 191
Leu Thr Leu Gly Gly Tyr Lys Thr Gly Thr Thr Ser Thr Ser Val Asn
        50                  55                  60

TTT ACA GAT GCC GCG GGT GAT CCC ATG TAC TTA ACA TTT ACT TCT CAG 239
Phe Thr Asp Ala Ala Gly Asp Pro Met Tyr Leu Thr Phe Thr Ser Gln
    65                  70                  75

GAT GGA AAT AAC CAC CAA TTC ACT ACA AAA GTG ATT GGC AAG GAT TCT 287
Asp Gly Asn Asn His Gln Phe Thr Thr Lys Val Ile Gly Lys Asp Ser
80                  85                  90                  95

AGA GAT TTT GAT ATC TCT CCT AAG GTA AAC GGT GAG AAC CTT GTG GGG 335
Arg Asp Phe Asp Ile Ser Pro Lys Val Asn Gly Glu Asn Leu Val Gly
                100                 105                 110

GAT GAC GTC GTC TTG GCT ACG GGC AGC CAG GAT TTC TTT GTT CGC TCA 383
Asp Asp Val Val Leu Ala Thr Gly Ser Gln Asp Phe Phe Val Arg Ser
            115                 120                 125

ATT GGT TCC AAA GGC GGT AAA CTT GCA GCA GGT AAA TAC ACT GAT GCT 431
Ile Gly Ser Lys Gly Gly Lys Leu Ala Ala Gly Lys Tyr Thr Asp Ala
        130                 135                 140

GTA ACC GTA ACC GTA TCT AAC CAA TAATCCATA TAGATAATAG ATAAAGGAGG 484
Val Thr Val Thr Val Ser Asn Gln
    145                 150
```

GCTATTATGC CCTCCTTTAA TATTTATGAA TTATCCTACT TTGAGCCTAA CCGTCGAC 542

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 151 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ala Pro Ala Asp Leu Thr Ala Ser Thr Thr Ala Thr Ala Thr Leu Val
1 5 10 15

Glu Pro Ala Arg Ile Thr Ile Thr Tyr Lys Glu Gly Ala Pro Ile Thr
20 25 30

Ile Met Asp Asn Gly Asn Ile Asp Thr Glu Leu Leu Val Gly Thr Leu
35 40 45

Thr Leu Gly Gly Tyr Lys Thr Gly Thr Thr Ser Thr Ser Val Asn Phe
50 55 60

Thr Asp Ala Ala Gly Asp Pro Met Tyr Leu Thr Phe Thr Ser Gln Asp
65 70 75 80

Gly Asn Asn His Gln Phe Thr Thr Lys Val Ile Gly Lys Asp Ser Arg
85 90 95

Asp Phe Asp Ile Ser Pro Lys Val Asn Gly Glu Asn Leu Val Gly Asp
100 105 110

Asp Val Val Leu Ala Thr Gly Ser Gln Asp Phe Phe Val Arg Ser Ile
115 120 125

Gly Ser Lys Gly Gly Lys Leu Ala Ala Gly Lys Tyr Thr Asp Ala Val
130 135 140

Thr Val Thr Val Ser Asn Glu
145 150

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 542 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..7

(ix) FEATURE:
(A) NAME/KEY: misc_recomb
(B) LOCATION: 1..6
(ix) FEATURE:
(A) NAME/KEY: misc recomb
(B) LOCATION: 536..541
(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 21..530
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
G AGC TCG TAGGAGGTAA TAT ATG AAA AAA ATC AGT TCC GTT ATC GCC ATT 50
  Ser Ser                Met Lys Lys Ile Ser Ser Val Ile Ala Ile
    1                      1               5                  10

GCA TTA TTT GGA ACT ATT GCA ACT GCT AAT GCG GCA GAT TTA ACT GCA  98
Ala Leu Phe Gly Thr Ile Ala Thr Ala Asn Ala Ala Asp Leu Thr Ala
                  15                  20                  25

AGC ACC ACT GCA ACG GCA ACT CTT GTT GAA CCA GCC CGC ATC ACT CTT 146
Ser Thr Thr Ala Thr Ala Thr Leu Val Glu Pro Ala Arg Ile Thr Leu
             30                  35                  40

ACA TAT AAG GAA GGC GCT CCA ATT ACA ATT ATG GAC AAT GGA AAC ATC 194
Thr Tyr Lys Glu Gly Ala Pro Ile Thr Ile Met Asp Asn Gly Asn Ile
45                  50                  55

GAT ACA GAA TTA CTT GTT GGT ACG CTT ACT CTT GGC GGC TAT AAA ACA 242
Asp Thr Glu Leu Leu Val Gly Thr Leu Thr Leu Gly Gly Tyr Lys Thr
     60                  65                  70

GGA ACC ACT AGC ACA TCT GTT AAC TTT ACA GAT GCC GCG GGT GAT CCC 290
Gly Thr Thr Ser Thr Ser Val Asn Phe Thr Asp Ala Ala Gly Asp Pro
 75                  80                  85                  90

ATG TAC TTA ACA TTT ACT TCT CAG GAT GGA AAT AAC CAC CAA TTC ACT 338
Met Tyr Leu Thr Phe Thr Ser Gln Asp Gly Asn Asn His Gln Phe Thr
                  95                 100                 105

ACA AAA GTG ATT GGC AAG GAT TCT AGA GAT TTT GAT ATC TCT CCT AAG 386
Thr Lys Val Ile Gly Lys Asp Ser Arg Asp Phe Asp Ile Ser Pro Lys
             110                 115                 120

GTA AAC GGT GAG AAC CTT GTG GGG GAT GAC GTC GTC TTG GCT ACG GGC 434
Val Asn Gly Glu Asn Leu Val Gly Asp Asp Val Val Leu Ala Thr Gly
        125                 130                 135

AGC CAG GAT TTC TTT GTT CGC TCA ATT GGT TCC AAA GGC GGT AAA CTT 482
Ser Gln Asp Phe Phe Val Arg Ser Ile Gly Ser Lys Gly Gly Lys Leu
    140                 145                 150

GCA GCA GGT AAA TAC ACT GAT GCT GTA ACC GTA ACC GTA TCT AAC CAA 530
Ala Ala Gly Lys Tyr Thr Asp Ala Val Thr Val Thr Val Ser Asn Gln
155                 160                 165                 170
```

TAATCCATAT AG 542

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ser Ser
1

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 170 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Lys Lys Ile Ser Ser Val Ile Ala Ile Ala Leu Phe Gly Thr Ile
1 5 10 15

Ala Thr Ala Asn Ala Ala Asp Leu Thr Ala Ser Thr Thr Ala Thr Ala
20 25 30

Thr Leu Val Glu Pro Ala Arg Ile Thr Leu Thr Tyr Lys Glu Gly Ala
35 40 45

Pro Ile Thr Ile Met Asp Asn Gly Asn Ile Asp Thr Glu Leu Leu Val
50 55 60

Gly Thr Leu Thr Leu Gly Gly Tyr Lys Thr Gly Thr Thr Ser Thr Ser
65 70 75 80

Val Asn Phe Thr Asp Ala Ala Gly Asp Pro Met Tyr Leu Thr Phe Thr
85 90 95

Ser Gln Asp Gly Asn Asn His Gln Phe Thr Thr Lys Val Ile Gly Lys
100 105 110

Asp Ser Arg Asp Phe Asp Ile Ser Pro Lys Val Asn Gly Glu Asn Leu
115 120 125

Val Gly Asp Asp Val Val Leu Ala Thr Gly Ser Gln Asp Phe Phe Val
130 135 140

Arg Ser Ile Gly Ser Lys Gly Gly Lys Leu Ala Ala Gly Lys Tyr Thr
145 150 155 160

Asp Ala Val Thr Val Thr Val Ser Asn Gln
165 170

(2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

TCGCCCGGGA ATTCCGAACA TAAATCGGTT CAGTGGCC 38

(2) INFORMATION FOR SEQ ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GGCGTATTCC TCGCTAGCAA TGTTTAACG 29

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii) HYPOTHETICAL: NO
(iii) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

ATCGTTAAAC ATTGCTAGCG AGGAATACGC C 31

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOTHETICAL: NO
(iii)ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A)ORGANISM: Yersinia pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

GATAGATCTG TCGACTGAAC CTATTATATT GCTTCGCGC 39

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOPTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE: (A) ORGANISM: Yersinia Pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTAGGAGCTC CCGCAGATTT AACTGCAAGC 30

(2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOPTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE: (A) ORGANISM: Yersinia Pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GATCGAGCTC GTAGGAGGTA ATATATGAAA 30

(2) INFORMATION FOR SEQ ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: DNA (genomic)
(iii)HYPOPTHETICAL: NO
(iii)ANTI-SENSE: NO
(vi) ORIGINAL SOURCE: (A) ORGANISM: Yersinia Pestis
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CAGGTCGAGC TCGTCGACCT ATATGGATTA TTGGT 35

CLAIMS.

1. Recombinant DNA that when incorporated into the DNA of a microorganism is capable of transforming that microorganism such that it is enabled to express a protein which produces a protective immune response against Yersinia pestis in a human or animal body when the microorganism is administered by oral route.

2. A plasmid capable of transforming a microorganism such that it is enabled to express a protein which produces a protective immune response against Yersinia pestis in a human or animal body when the microorganism is administered by oral route.

3. A microorganism comprising recombinant DNA, or a plasmid comprising recombinant DNA, whereby it is enabled to express a protein which produces a protective immune response against Yersinia pestis in a human or animal body when administered by oral route.

4. A microorganism as claimed in Claim 3 characterised in that it is a human or animal gut colonising microorganism.

5. Recombinant DNA, a plasmid or a microorganism as claimed in any one of claims 1 to 4 wherein the transformed microorganism maintains its ability to colonise the human or animal gut.

6. Recombinant DNA, a plasmid or a microorganism as claimed in Claim 1, 2, 3, 4 or 5 wherein the protein which produces the immune response comprises all or part of the F1 protein of Yersinia pestis.

7. Recombinant DNA comprising a DNA sequence as described in SEQ ID No 4.

8. Recombinant DNA as claimed in claim 1 comprising the F1 operon of Yersinia pestis including the caf1R, caf1M, caf1A and caf1 gene subunits.

9. Recombinant DNA as claimed in claim 8 wherein the F1 operon is derived by PCR amplification of Yersinia pestis template DNA using primer pairs of SEQ ID No 11 and 12 and SEQ ID No 13 and 14 to produce two discrete fragments, these fragments digested using Nhe1 and then joined to provide a single F1 operon containing fragment.

10. A plasmid comprising recombinant DNA as claimed in claim 8 or 9.

11. A plasmid as claimed in claim 10 being a low copy number plasmid.

12. A plasmid as claimed in claim 10 or 11 wherein the plasmid is pLG339 or pBRD1084.

13. A plasmid as claimed in Claim 2 comprising a DNA sequence as described in SEQ ID No 4.

14. A plasmid as claimed in Claim 2 or Claim 13 characterised in that it comprises a lacZ promoter upstream of a sequence encoding for all or part of the F1 antigen.

15. A plasmid as claimed in Claim 14 characterised in that it comprises a pUC18 vector that has all or part of the caf1 sequence inserted downstream of the lacZ promoter.

16. A microorganism as claimed in Claim 3 or 4 containing recombinant DNA comprising a DNA sequence as described in SEQ ID No 4 or as claimed in claim 8 or 9.

17. A microorganism as claimed in Claim 3 or 4 containing a plasmid as claimed in any one of claims 7 to 15.

18. A microorganism as claimed in Claim 3, 4, 16 or 17 being an attenuated microorganism not capable of causing disease in humans or animals.

19. A microorganism as claimed in Claim 3, 4, 10, 11 or 12 being an aro A and/or aro C mutant.

20. A microorganism as claimed in Claim 18 or 19 being a Salmonella.

21. A microorganism as claimed in Claim 20 being a Salmonella typhimurium or a Salmonella typhi.

22. A microorganism as claimed in any one of claims 3, 4, 18, 19, 20 or 21 wherein a recombinant DNA of claim 8 or 9 is integrated directly into the microorganisms chromosomal DNA.

23. A vaccine comprising a microorganism as claimed in any one of Claim 3, 4 or 16 to 22 together with a pharmaceutically acceptable carrier.

24. A recombinant DNA, plasmid, microorganism or vaccine according to any one of claims 1 to 23 as described in Example 1 or Example 4.

Fig.1.

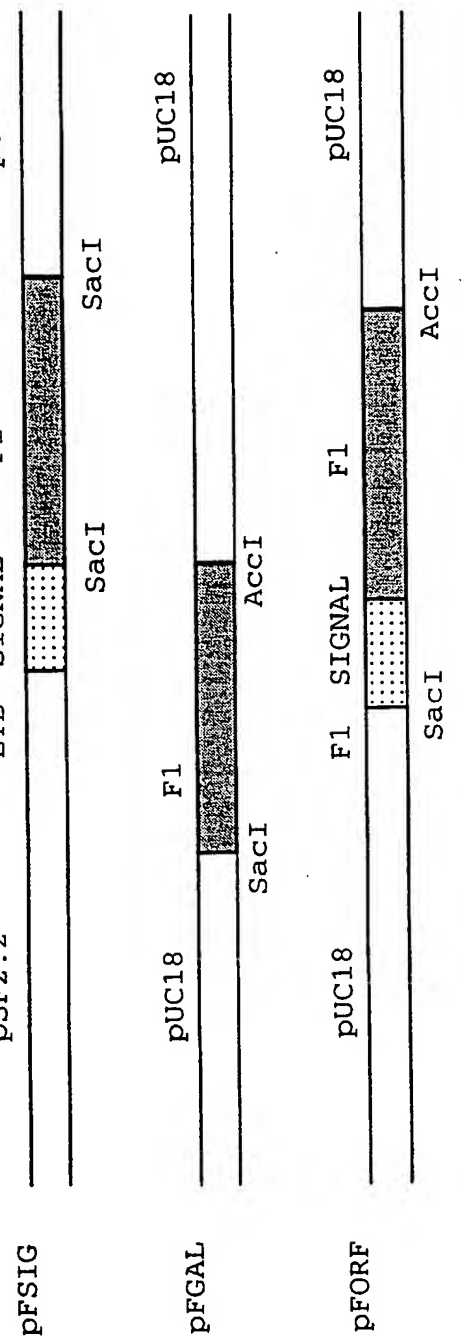

Fig.2.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C12N15/74 C12N1/21 A61K39/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AM.J.TROP.MED.HYG., vol.43, no.4, October 1990, USA pages 389 - 396 SIMPSON, W.J. ET AL. 'Recombinant capsular antigen (fraction 1) from Yersinia pestis induces a protective antibody response in Balb/C mice' cited in the application see Figure1; page 392, left-hand column, first paragraph; page 392, right-hand column, first and second paragraph --- -/--	1,2,5,6, 8-10,14, 15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 May 1995	29.05.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Alt, G

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FEBS LETTERS, vol.277, no.1,2, December 1990 pages 230 - 232 GALYOV, E.E. ET AL. 'Nucleotide sequence of the Yersinia pestis gene encoding F1 antigen and the primary structure of the protein' see page 230, right-hand column, second paragraph; Figures 1 and 2	1,5,6
Y	WO,A,92 08486 (WASHINGTON UNIVERSITY) 29 May 1992 see page 12, last paragraph	1-24
Y	VACCINE, vol.10, no.2, 1992 pages 75 - 88 MCGHEE, J.R. ET AL. 'The mucosal immune system: From fundamental concepts to vaccine development' cited in the application see page 78, left-hand column, last paragraph; page 84, right-hand column, lines 25-65 - page 85, left-hand column, lines 1-11	1-24
A	EP,A,0 474 891 (BEHRINGWERKE AKTIENGESELLSCHAFT) 18 March 1992 cited in the application	1
A	ADV.EXP.MED.BIOL., vol.303, 1991, USA pages 169 - 184 BREY, R.N. ET AL. 'Oral delivery of antigens in live bacterial vectors'	1
E	INFECTION AND IMMUNITY, vol.63, no.2, February 1995 pages 563 - 568 OYSTON, P.C.F. ET AL. 'Immunization with live recombinant Salmonella typhimurium aroA producing F1 antigen protects against plague' see whole document	1-24

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9208486	29-05-92	AU-A-	9120491	11-06-92
		CA-A-	2095534	10-05-92
		CN-A-	1063416	12-08-92
		EP-A-	0556333	25-08-93
		JP-T-	6501849	03-03-94
		NZ-A-	240538	26-01-94
		US-A-	5387744	07-02-95
		US-A-	5294441	15-03-94
EP-A-0474891	18-03-92	AU-A-	8368991	12-03-92
		CA-A-	2050876	09-03-92
		CN-A-	1060498	22-04-92